Practical problems of diagnosing trichomoniasis in women

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SUMMARY Analysis of eight groups of data collected at varying intervals during a period of seven years showed fluctuations in the sensitivity of tests to diagnose trichomoniasis in women. The best results were obtained from fresh, correctly prepared Diamond's medium, Feinberg-Whittington's medium, and modified Squires and McFadzean's medium (which showed 82% to 94% relative sensitivity). Poor results were identified consistently in commercially prepared Bushby medium from one source (40% relative sensitivity) and in a batch of commercially prepared Squires and McFadzean's medium from which chloramphenicol had been omitted (23% relative sensitivity). Examination of wet film, culture, and exfoliative cytology stained by Papanicolaou's method were shown to be indispensable for auditing the performance of each test and to maintain the quality of a diagnostic service.

The problems of diagnosing trichomoniasis in men are well known, but the need to monitor the sensitivity of the tests in women is less widely appreciated. Moreover, published reports on the reliability of individual tests can be misleading in that they may not be repeatable in busy clinics employing observers of mixed ability. Our own experiences of lapses in a routine service for a large high risk population illustrate the need for vigilance and quality control.

Patients and methods

Data were collected in this department as exercises in quality control at various times from December 1979 to May 1986. Specimens were all taken from women undergoing routine investigation for the diagnosis and exclusion of sexually transmitted infection. All had been tested for the presence of *Trichomonas vaginalis* by three methods, namely: exfoliative cervical cytology with Papanicolaou staining, wet film examined by light field or phase contrast microscopy, and culture in one of four media. The four media were commercially prepared Bushby's medium; modified Feinberg-Whittington's medium, prepared locally with chloramphenicol 50 mg/l, gentamicin 80 mg/l, and amphotericin 10 mg/l; modified Squires and McFadzean's medium commercially prepared with

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prepared locally with chloramphenicol 100 mg/l, gentamicin 80 mg/l, and amphotericin 10 mg/l.

Specimens for exfoliative cervical cytology were taken immediately after inserting the speculum, and

chloramphenicol 125 mg/l; and Diamond's medium⁷

Specimens for exfoliative cervical cytology were taken immediately after inserting the speculum, and samples for wet film and culture were taken from the posterior fornix with a cotton tipped swab after taking the specimens for cervical tests. Wet films were examined within 15 minutes by an experienced medical laboratory scientific officer working in the clinic. Cultures were incubated at 37°C in an incubator at the clinic and transferred to the laboratory. Incubation of cultures continued for 48 hours, and they were then examined by microscopy for the presence of trichomonads.

Cytology specimens, which were processed at the women's hospital, Birmingham, were taken routinely only once a year from each woman tested, according to clinic policy, and were not used primarily for diagnosing infection. For the purposes of this exercise we excluded women who had not been tested by all three methods.

The χ^2 test with Yates's correction was used to compare the eight groups of data.

Results

Table I shows the sensitivity of the three tests at various times using one of eight different media for culture. For simplicity, positive results are shown as percentages only.

Accepted for publication 27 June 1987

Table 1 Sensitivity (% positive results) of three tests (number in parentheses show percent positive in stated test only) performed on varying numbers of women at various times during seven years

Culture medium	No of specimens	Percentage positivity in:			
		Wet film	Culture in medium stated	Cytology	All three tests
Bushby's*	176	70 (6)	40 (6)	85 (15)	22
Feinberg-Whittington's	82	73 (0)	93 (12)	79 (6)	63
Squires and McFadzean's†	61	21 (2)	82 (26)	67 (15)	13
Squires and McFadzean's	47	64 (0)	94 (21)	70 (4)	53
Squires and McFadzean's1	13	77 (23)	23 (0)	77 (23)	23
Diamond's	74	47 (1)	93 (20)	72 (5)	38
Squires and McFadzean's§	32	72 (6)	84 (9)	69 (3)	44
Diamond's§	99	67 (0)	92 (16)	74 (5)	54

^{*}Time delay between manufacture and use of this commercially prepared culture medium.

§Phase microscopy used instead of light microscopy.

CYTOLOGY

When complemented by other acceptable tests, the "false positive" results ranged from 3% to 6%. Many of these were later shown by other tests to be true positive results, but a precise analysis was beyond the scope of this report. Higher incidences of "false positive" results, which ranged from 15% to 23%, were associated with poor positivity in another test and a lower incidence of concurrence between the three tests.

WET FILM

The poorest sensitivity of this test was when positive results were obtained in only 21% of patients. This resulted from contamination of saline dispensers with glutaraldehyde, which had been used to clean the dispensers to eliminate yeasts. The difficulty was overcome by using saline "spotted" directly on to the slide from 1 ml ampoules. In a small series of experiments 0.002–0.004% glutaraldehyde was shown to immobilise T vaginalis in 10 minutes.

There was a fairly wide range of sensitivity in the rest of these tests, with positive results in 47% to 77% of patients. These figures related not only to differences from results of the other tests used, but also to the varying experience of laboratory staff. However, improvement in positive results because of the change from light to phase contrast microscopy (from 47% up to 67% of patients positive) in the comparison with Diamond's medium was probably real (p < 0.01). There was a similar but not significant improvement with Squires and McFadzean's medium (from 64% to 72%).

CULTURE

Poor results were obtained from two types of commercially prepared medium. The first of these was a Bushby medium that we were unable to obtain fresh

because of the structure of the distribution chain. The positivity when using this medium was only 40%.

A commercially prepared Squires and McFadzean's medium gave positive results in 84% to 94% of patients, but one batch gave only 23% positivity, and this poor performance was attributed to the omission of chloramphenicol. Good results were obtained from a modified Feinberg-Whittington's medium and Diamond's medium, which gave positive results in 92% to 93% of patients. Both these media were prepared in our own laboratory and could be made fresh each week to suit fluctuating seasonal demands.

Discussion

Although there are comprehensive critical reviews of the problems of diagnosing trichomoniasis in women, ⁸⁹ many textbooks and publications ignore or underplay the need for quality control. Nevertheless, we have shown that monitoring test results is essential to maintain and improve the quality of a routine diagnostic service.

Most workers agree that culture is the most sensitive test, ^{2 10-12} yet there is no universal agreement about which media are best. Conflicting results have been obtained in studies comparing media and even using the same medium at different times. ^{10 13 14} Cox and Nicol showed that the growth rates of different strains of *T vaginalis* varied between experiments and with the age of the medium. ¹⁵ This type of consideration, with the other possible sources of error shown in table 2, may account for some of the discrepancies between published reports.

Despite evidence supporting the routine use of culture, examination of the wet film still remains the sole screening test in many gynaecology and genitourinary clinics. Hess⁹ and more recently Smith¹² also recommended culture for *T vaginalis* only if the

[†]Saline dispensers contaminated by glutaraldehyde.

Chloramphenicol omitted from this batch of commercially prepared culture medium.

Table 2 Possible sources of error in three methods used to diagnose trichomoniasis in women shown by various workers

Poor sample¹⁴
Scanty organisms
Incomplete examination^{14 16}
Misinterpretation³
Wet film
Poor sample collection (too little or too much)⁹
Scanty organisms¹¹
Delay in examination (causing cooling or dessication)¹⁴
Hasty microscopy^{11 16}
Suboptimum conditions for microscopy (dark field or phase

contrast preferable)8
Toxic dispersal medium or douche11

Presence of blood¹⁵ Culture

Cytology

Scanty organisms^{1 15}
Medium not fresh¹⁵
Faulty pH adjustment (5·5 to 6·0 is optimum)⁸
Incubation temperature not optimum⁷
Suppression of growth by bacteria or fungi¹
Some strains fail to grow (possible nutritional defect of medium)¹⁵
Examinations of cultures too early to too late (48 hours is optimum)¹³
Delay in subculture from transport medium⁸

examination of the wet film gave a negative result. The application of such a procedure in the present study would have missed the diagnoses in 3–23% of cases where the organism was detected by exfoliative cytology alone. Thin et al showed that Papanicolaou smears were as good as wet film or cultures in diagnosing trichomoniasis, but for the highest sensitivity they recommended the simultaneous use of all three methods. We have not only confirmed this observation, but have also shown that this contributes to good quality control.

Studies that have been performed to assess the usefulness of repeat testing in detecting gonorrhoea in women support the use of at least two tests to exclude or establish the diagnosis. ¹⁷¹⁸ Erikson and Wanger showed that second and third examinations with wet film and culture, performed at weekly intervals, detected 6.0% and 2.4%, respectively, of 164 cases of trichomoniasis in women. ¹⁹ In addition to increasing diagnostic yields, repeat testing may also be used to identify errors in previous tests in a routine service and is desirable to evaluate new techniques.

Nagel and Kunz studied the performance of 46 different commercially prepared media during eight months.²⁰ They concluded that the primary responsibility for quality control should rest with the manufacturer or vendors, rather than with the purchaser or users, who may not have the necessary expertise or resources. Our experience suggests that this recommendation is unrealistic and that the diagnostic laboratory, in conjunction with the clinics that use it most, should be responsible for monitoring performance.

It can be argued that the simultaneous use of three

methods is not cost effective, but in fact many users of the service will be relying exclusively on one method, and in general that method will normally be culture. Where wet film is being used for immediacy, culture for superior results, and cytology for proper indications, however, that information should be used constructively for quality control.

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